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VENOM AND ENVENOMATION OF IRANIAN BLACK WIDOW SPIDER, *LATRODECTUS*

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Abstract – Spider *Latrodectus* (black widow) has a wide distribution around the world. The bite of this spider due to it's lethal neurotoxic venom is medically important. In this study two species of spiders *Latrodectus palidus* and *Latrodectus dahli* were hunted from the central provinces of Iran like Saveh, Ghazwin (Boinzahra), Teheran (Savojbolagh) in large number and their venom was extracted by homogenization of dissected venom glands and centrifugation of homogenate, separation of filtrate and lyophilization of extracted venom. The amount of freeze dried venoms were 0.7 grams for *L. palidus* and 0.8 grams for *L. dahli*. The toxicity of the venoms were tested in 18-20 grams Albino mice through tail vein according to Spearman and Karber method. The LD_{50} of so called venoms were 13.9±0.2 microgram for *palidus* and 19.8 microgram for *dahli.* Electrophoresis results from SDS-Page showed 10 bands for *Palidus* and 12 bands for *Dahli* with molecular weight ranging from 10 to 110 kilodalton.

Key words : Spider, *Latrodectus palidus, Latrodectus dahli*, venom, toxicity, LD_{50}

INTRODUCTION

Latrodectus is a genus of spider in the family Theridiidae which contains 32 recognized species. The medically important genus are *Latrodectus, Loxosceles,Phoneatria* and *Atrax* which morbidity and mortality in human (WHO, 1981). The genus *Latrodectus* are known with "black widow" common name. due to a behavior seen in some species in which the female eats the male after mating (Breene *et al*, 1985). Unlike most species of widow spiders, which are dark in colouration. *Latrodectus pallidus*, the white widow spider is light-colored, with coloration ranging from beige to white with darker legs. The spider lacks the bright red markings found on other widow spiders such as the black widow.

The spiders in the family Theridiidae have a wide distribution within the world. Widow spiders can be found on every continent of the world like north and south America, north and south Africa, western Asia and Australia (White, 1985). All spiders of genus *Latrodectus* have a venomous bite which can injure human. The white widow bite is medically significant and can kill children and the infirm(Wikipedia, 2008).

The venom of the black widow is an oily yellow fluid that cause a general release of neurotransmitters at synaptic junctions, and so the clinical manifestation of black widow bite are primarily neurological. The insertion of the female fangs produces mild sharp pain. Tissue reactions at the site of bite are minimal , but they may include redness, mild swelling and itching. Within 30 to 60 minutes the victim experiences severe pain in local muscle group. Severe cramp may spread throughout the body. In a typical case, the victim experience unremitting, cramping pain of extremities, thorax, back, abdomen, and groin areas. Pain and cramping peaks at 2 to 3 hours and may debilitate the victim for 24 to 36 hours. Other symptoms include hypertension, profuse perspiration, nausea, fatique, shock and coma, which may be fatal. Death has been reported in less than 1% of documented black spider bite cases. Healthy individuals usually recover quickly and completely (Blackman, 1995; Kunkel, 1984; King, 1990).

Toxic components of *Latrodectus* spider venom called latrotoxin, shown to contain a toxin specific for mammals (α-latrotoxin) and at least five insectospecific toxins (insecticidal toxins), termed as α, β, γ, δ and ε-latroinsectotoxins and one toxin affecting Crustaceans (α-latrocrustatoxin) (Rohow *et al*, 2006).

These toxins stimulate massive release of neurotrnsmitters from nerve terminals and act (i)- by binding to specific receptors, some of which mediate an exocytotoxic signal and (ii)- by inserting themselves into the membrane and forming ion-permiable pores. Except for members of latrotoxin family that were commonly considered as the primary components of venom, several other special enzymes and proteins were detected such as protease, phosphatase, lysozyme, inhibitory protein and so on (Duan *et al*, 2006).

Onset of clinical signs usually occurs during the first 8 hours post envenomation, the condition is extremely painful

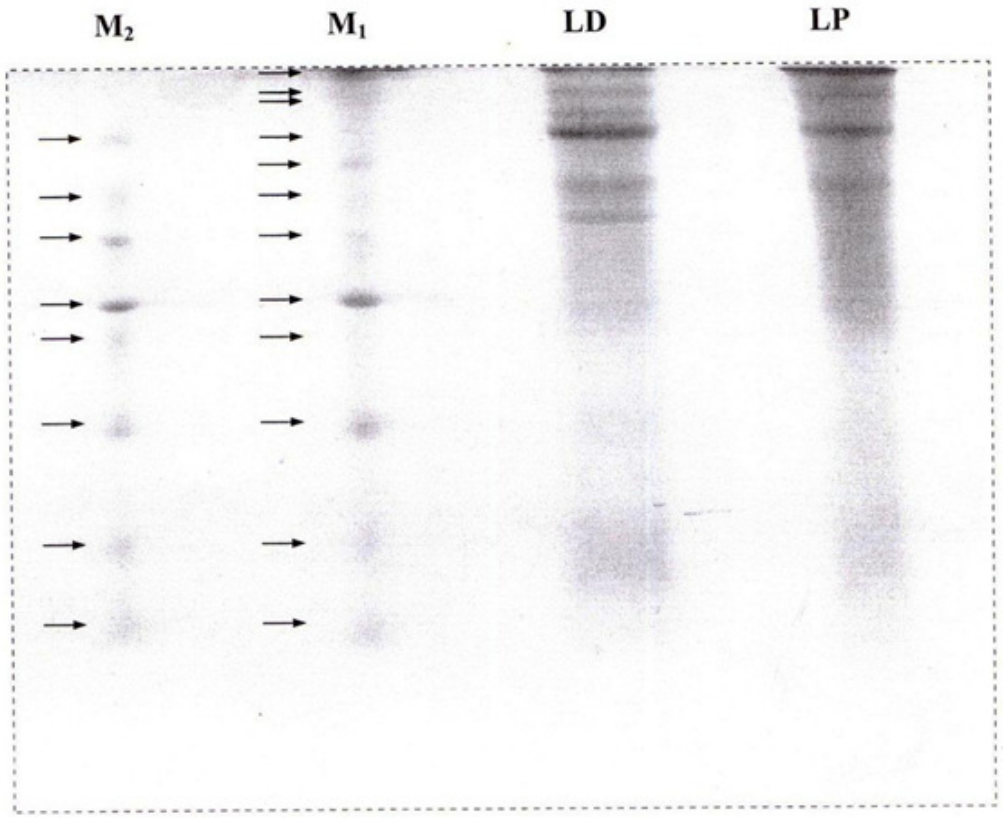

Fig.1: SDS-PAGE electrophoresis pattern of spider venom

LP: crude venom of *Latrodectus pallidus;* **LD:** crude venom of *Latrodectus dahlia;* $\mathbf{M_1}$**:** wide range Molecular weight marker (6.5, 14.2, 20, 24, 29, 36, 45, 55, 66, 116, 200 KD); $\mathbf{M_{2:}}$ Low range Molecular weight marker (6.5, 14.2, 20, 24, 29, 36, 45, 55, 66 KD)

in moderate to severe envenomation. Abdominal rigidity without tenderness is a hallmark sign of of *Latrodectus* envenomation in cats, paralytic signs may occur early and are particularly marked. Hypertension is a significant threat (Peterson, 2006).

Until 1942, only 21 species and 8 subspecies were reported from this genus. During 1959-1966 six species of *Latrodectus* were reported in middle east and 2 species *L. dahli* and *L. pallidus* were reported from southern-Iran. In 1994 *Latrodectus tredecimguttatus* was collected from suburb of Mashhad city in Khorasan province north-eastern Iran (Godarzi *et al*, 1974). Then in order to study spiders in southern- Khorasan, Mirshamsi Kakhaki reported 3 species *Latrodectus tredecimguttatus, L. dahli* and *L. pallidus* (Mirshamsi Kakhaki *et al*, 2005). Finally in 1991 (Akbari *et al*) collected and studied 2 species*, L. dahli and L. pallidus* from Eshtehard and Svojbolagh in Tehran province and from Boin Zahra, south-eastern part of Ghazvin province and Saveh region in north part of central province (unpublished report).

In this study, large number of spider *L. dahli* and *L. pallidus* were collected from Tehran and central provinces and their venom was extracted by homogenization of venom glands. The extracted venom was freezedried and

Table 1 : Comparison of protein fractions in the venoms of Spiders *L. dahli* and *L. pallidus*.

Band number	MW of bands(KD)	*L.Dahli*	*L.Pallidus*
1	200	-	-
2	115	+	+
3	90	+	+
4	66	+	+
5	55	-	-
6	45	+	-
7	43	-	+
8	39	+	-
9	34	+	-
10	32	+	+
11	30	+	+
12	28	+	+
13	25	+	+
14	19	+	+
15	10	+	+

LD_{50} of the venoms of 2 species were determined in 18-20 gram Albino mice by Spearman and Karber method. For evaluation of different proteinic fractions the venoms were analyzed by SDS-PAGE gel electrophoresis. The molecular weight of the bands were estimated by comparison with specific markers.

MATERIALS AND METHODS

Spiders *L. dahli* and *L. pallidus* were collected from Nazarabad, Eshtehard and Najmabad places around Saveh city, by direct observation in fields and gardens. The mature female spiders were collected and transferred to reference laboratory at Razi Research Vaccine and Serum Institute, where their venom glands were removed surgically from cephalotorax region. Dissected venom glands were homogenized(Dr. Hielscher; UP 200 H, Germany) in cold distilled water, then the homogenate was centrifuged (ALC-PK 131 R; UK) at 8000 rpm for 20 minutes at 4^{oc} the supernatant was separated and freeze dried (Schnider; Germany). Weight of dried venom was about 70-80 mg for 100 spiders or 700-800 µg per one spider.

The LD_{50} (toxicity) of venoms was determined in 18-20 gram Albino mice by intravenous injection through tail vein according to Spearman and Karber (1956) method. 30 µg of each venom and 2 types of molecular weight marker (high and low molecular weight) were applied SDS-PAGE (Paya Pajohesh; Iran) in 13x18 cm plates at 150 v Voltage for 5 hours. Then the molecular weight of each resolved band was determined by comparison with specific protein markers.

RESULTS AND DISCUSSION

500 *L. pallidus* and 600 *L. dahli* spiders were collected from their habitats and their venoms were extracted by homogenization technique and freeze dried. The average extracted dry venom was 0.7 mg for each *L. pallidus* and 0.8 mg for each *L. dahli* spiders.

For determination of LD_{50}, different dilutions of *L. pallidus* venom containing 8, 10, 12.5, 15.6, 19.5 and 24.4 µg venom were injected to Albino mice. The LD_{50} of the venom of *L. pallidus* was 13.9 µg for each mouse or 0.62±0.01 mg/kgbody weight. For determination of LD_{50}, different dilutions of *L. dahli* venom containing 10, 12.5, 15.6, 19.5, 24.4 and 30.5 µg venom were injected to Albino mice. The LD_{50} of the venom of *L. dahli* was 19.8 µg for each mouse or 0.99±0.01 mg/kgbody weight.

Clinical signs of injected mice were studied, those animals which received higher amount of venom showed intense stretching of distal organs toward back, muscle cramp of chest, abdomen and legs and mortality in first minutes after injection. Those mice which received lower amount of venom did not show any sign and survived.

SDS-PAGE results showed 10 proteinic bands with molecular weight ranging from 10 to 115 KD for *L. pallidus* and 12 proteinic bands with molecular weight ranging from 10 to 115 KD for *L. dahli* as shown in table 1 there are some similarities between the fractions isolated from 2 spiders specially in 8 fractions with molecular weights of 115, 90, 66, 32, 30, 25, 19 and 10 KD which are present in the venoms of *L. dahli* and *L. pallidus.* 45, 39 and 34 KD fractions are only present in in *L. dahli* venom are absent in *L. pallidus* venom. Fraction with 43 KD molecular weight is only present in *L. pallidus* venom.

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